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14. ABSTRACT

In this study we propose to develop and validate pulmonary nodule elastometry imaging, a method complementary to CT that has the potential to increase the specificity of screening for early detection of lung cancer. We propose to address the need for greater specificity in lung cancer screening by characterizing a mechanical property of pulmonary lesions, specifically pulmonary nodule (PN) elasticity, in addition to standard anatomic features. We hypothesize that malignant and benign PN can be distinguished more specifically by different elasticities determined from 4D CT images. The specific aims of the study were the development of pulmonary nodule elastometry algorithms based on deformable image processing of 4D CT images and their validation in an animal model and in a retrospective review of over 200 4D CT scans from patients with small malignant pulmonary nodules previously treated with radiation in our department. We have successfully developed the algorithms, and in a first validation we have demonstrated proof of principles that elastometry can distinguish malignant PNs from surrounding lung tissue (a manuscript is submitted and under review/revision). The validation in animal models and the retrospective analysis of the human data is ongoing.

15. SUBJECT TERMS

Pulmonary nodule elastometry, 4DCT, deformable image registration, Jacobian, lung cancer, lung cancer screening

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REVISED REPORT

Contract number: W81XWH-12-1-0287

Title: Improving the Diagnostic Specificity of CT for Early Detection of Lung Cancer: 4D CT-Based Pulmonary Nodule Elastometry

Principal Investigator: Billy W Loo Jr, MD PhD

Introduction:

In this project we are addressing a shortcoming of existing lung cancer screening methods by developing a CT based method of characterizing a mechanical property of pulmonary lesions, specifically tissue elasticity (stiffness) that should have a higher specificity than purely anatomic low-dose CT. It is the aim of the proposed study to decrease the false positive rate of CT screening by analyzing the mechanical properties of suspicious appearing pulmonary nodules during CT screening. We hypothesize that malignant pulmonary nodules are less elastic (stiffer) than benign nodules and that this difference in elasticity can be used to differentiate cancerous from benign nodules, which would help to decrease the false positive rates of CT screening. A measure of elasticity can be derived from high-resolution 4-dimensional computed tomography (4D CT) using deformable image registration algorithms. Unlike conventional 3D CT imaging that results in a static image of the scanned anatomy, 4D CT incorporates also the temporal changes of the anatomy caused by respiratory motion, yielding a CT 'movie' that allows the evaluation of tumor motion and the calculation of the elasticity.

Body:

Specific Aim 1. ***Development of deformable image algorithms for processing the 4D CT images to determine the elasticity of malignant and benign pulmonary nodules.*** *(**Dr. Maxim**, Tasks 1, months 1 – 8)*

Task 1. Development of the software for deformable image registration, analysis of the DVF and the calculation of the elasticity parameter (Matlab).

The software will be developed using the mathematical package Matlab (The Mathworks Inc., Natick, MA). Two deformable image registration algorithms will be used (DIR^{vol} and a method based on optical flow, DIR^{OF}). The resulting

*displacement vector fields will be analyzed and an elasticity parameter for the pulmonary nodules will be calculated (**Dr. Maxim**, months 1 – 8).*

Status (Task 1):

A manuscript describing our algorithm and its validation has been submitted to 'Radiotherapy and Oncology' (Green Journal) and is attached to this report. We have submitted a second round of responses to reviewers' comments and anticipate that it will now be accepted for publication after having addressed all concerns.

Specific Aim 2: Validate our method in rat models of human lung cancer and benign inflammatory lesions. *(**Dr. Maxim**, Tasks 2-4, months 3 – 24)*

Task 2. Preliminary experiments: Establish optimal protocol for the benign pulmonary model (granulomatous inflammation) and study growth kinetics.

*2a. Purchase animals: Rowett rats, A549 and SK-MES-1 cells from American Tissue Culture Collection (ATCC), carbon nanotubes (catalogue number 900–1501, lot GS1801), SES research (Houston, TX) and necessary culturing media. (**Dr. Maxim**, months 1-3)*

*2b. Inoculate 15rats (Rowett nude rats) with carbon nanotubes and follow with serial MicroCT measurements to study growth kinetics to establish the time for nodule development to reach desired size. (**Dr. Maxim**, 15 rats total, months 3 – 6)*

Task 3. Grow orthotopic model of lung cancer and benign lesions and follow with serial MicroCT imaging: preliminary experiments to establish protocol and optimize software

*3a. Inoculate 10 rats with orthotopic human lung cancer cells (A549, left lung) and carbon nanotubes (right lung) (**Dr. Maxim**, months 7-9)*

*3b. Acquire CT images at peak-inhale and peak-exhale using a small animal ventilator (**Dr. Maxim**, month 9-10)*

*3c. Analyze CT images and derive elasticity parameter and optimize software if necessary. (**Dr. Maxim**, month 10)*

Task 4. Grow orthotopic model of lung cancer and benign lesions and follow with serial MicroCT imaging, analyze data

*4a. Inoculate remaining 40 rats (A549 cells, left lung in Rowett nude rats) and follow with CT imaging at peak-inhale and peak-exhale (**Dr. Maxim**, months11-13)*

*4b. Perform simplified analysis: Delineate malignant and benign pulmonary nodules and measure volumes at peak-inhale and peak-exhale. Derive elasticity parameter based on the ratio of the volumes. (**Dr. Maxim**, months 14-15)*

*4c. Analyze acquired CT images and derive elasticity parameter by analyzing the displacement vector fields and perform statistical analysis. (**Dr. Maxim**, months 16-18)*

*4d. Repeat experiments and analysis with second cancer cell line (SK-MES-1), 50 Rowett rats, (**Dr. Maxim**, months 18-23)*

*4e. Publish animal study results (**Dr. Maxim**, month 24)*

Status (Tasks 2, 3, 4): Due to ongoing repairs and upgrades of the GE-MicroCT scanner, our proposed experiments are being delayed again. The capability of acquiring 4DCT images is hampered by a defect in the GE-MicroCT scanner. Given this delay, we asked the DoD for a one-year no-cost extension, which was recently approved.
We were able to to successfully generate our benign model using the talc (instead of the proposed carbon nanotubes) as shown in Figure 1.

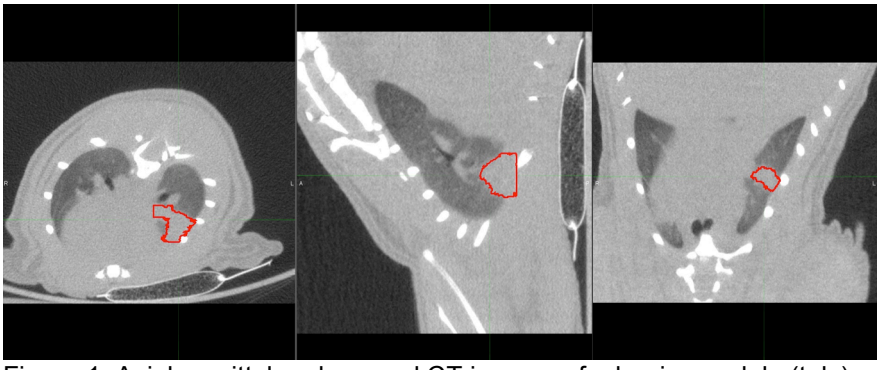

Figure 1: Axial, sagittal and coronal CT images of a benign nodule (talc), delineated in red in the left lung of a rat.

As soon as the capability of acquiring 4DCT images is restored on the GE-MicroCT scanner, we are ready to acquire and analyze the mechanical properties of the tumors and the benign tissue in accordance with the proposed method.

The reliability of the micro-CT scanner remains an issue that has slowed progress. A major repair is scheduled by March 2015 that will hopefully improve the performance of the scanner substantially. Nevertheless, we have continued to make progress on experiments and now have acquired images of multiple animals both with benign nodules (talc granulomas) as well as malignant orthotopic lung tumors.

Figure 2 shows respiratory-gated micro-CT images of an orthotopic tumor implanted in the lung of a nude rat, showing the relative change in volume between the tumor and the lung as a measure of stiffness.

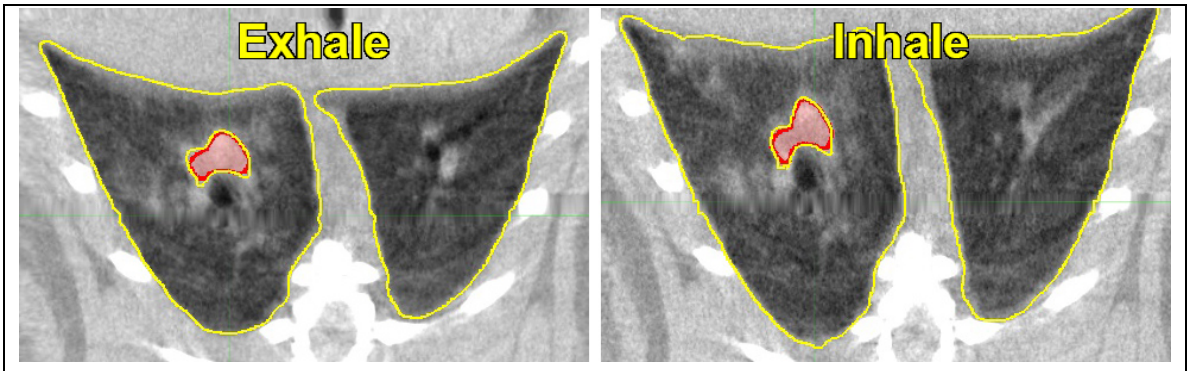

Figure 2: Coronal images of an orthotopic lung tumor in a rat model (outlined in red) in micro-CT images gated at exhale and inhale, demonstrating the relative volume change between the tumor and lung.

Analysis of the malignant tumor and benign talc models is ongoing. Preliminarily, there may be little difference in elasticity between these nodule types, indicating that talc granulomas may be very stiff. As such, we have expanded the study to include another benign tumor model, injected matrigel, to demonstrate the ability of the imaging method to distinguish nodules with a range of elasticities.

Specific Aim 3: Validate our method in a retrospective review of over 200 4D CT scans from patients previously treated in our department. *(**Dr. Loo**, Task 5 months 1 – 20)*

Task 5. Analyze approximately 200 4D CT images from previously treated patients and patients recruited within the funding period.

5a. De-archive all previously acquired thoracic 4D CT scans and identify suitable patients for the study. Our institutional data (all 4D CT scans) are currently stored on DVD's. Data will be de-archived and suitable lung cancer patients

*(patients with benign and malignant pulmonary nodules) will be identified. (**Dr. Loo**, months 1 – 3)*

5b. *Identify benign and malignant pulmonary nodules to be included in the analysis and delineate nodules at each respiratory phase. (**Dr. Loo**, month 4)*

5c. *Perform simplified analysis by calculating the ratio of the volumes with respect to peak-inhale. (**Dr. Loo**, months 5-8)*

5d. *Analyze all 4D CT images and derive elasticity parameter by analyzing the displacement vector fields and perform statistical analysis (**Dr. Loo**, months 9-15)*

5e. *Analyze data from new patients acquired during the award period (**Dr. Loo**, months 15-18).*

5f. *Publish human study results (**Dr. Loo**, months 19-20)*

Status (Task 5): We continue to de-archive and analyze more patients. We have identified several patients with benign nodules (in addition to malignant nodules that were treated in our department). From those patients with benign nodules, 30% showed strong motion artifacts in 4DCT that aggravated the analysis. 23% of thus far analyzed patients have very small benign nodules. With the acquired CT resolution, the nodules are comprised of a few voxels. Deformable image registration for objects with such few voxels is inaccurate and 'noisy', thus the data will have limited value. Dr. Loo has started with the delineation of the benign and malignant nodules. Data will be processed and analyzed shortly.

Because all patients in our database have been treated for malignant nodules, we have ample data on the malignant nodules, and the main challenge is identification of patients who happen to have benign nodules as well. We have now identified 47 patients in this patient cohort with nodules that were not treated because they were potentially benign. Because these nodules are most often observed by imaging (CT and/or PET) surveillance rather than biopsied with invasive procedures, there must be sufficient follow up to be confident that a nodule is benign. Of the 47 patients with potentially benign nodules, 34 have sufficient follow up to make a determination of malignant status. We are now engaged in careful comparisons of the serial imaging data to confirm whether all of these nodules are in fact benign. As described above, of the subset of

confirmed benign nodules so far, technical limitations include 4DCT artifacts and insufficient size to make accurate volume change determinations for several of them.

The painstaking imaging review required to identify and confirm benign nodules of sufficient size and image quality to be analyzable has slowed progress compared to the original projected timeline. However, we have now recruited an additional resident (Dr. Jennifer Shaffer) with both radiation oncology and diagnostic radiology training and who is on a dedicated 10-month research block to assist with the analysis. We anticipate that identification and characterization of all candidate nodules will be completed by the end of March 2015. As the image analysis methodology is now well established, the determination of elasticity parameters should be completed by the end of April 2015.

As an additional strategy in case the number of confirmed benign nodules is inadequate to make a strong statistical comparison, we are performing an additional analysis on patients with malignant tumors to determine if CT pulmonary nodule elastometry can characterize the aggressiveness of malignant nodules, as opposed to distinguishing benign from malignant. This is an equally relevant question in the context of CT screening for lung cancer because it similarly addresses the question of which nodules require more urgent biopsy versus imaging follow up. We have identified 60 patients with malignant tumors whose elasticity has already been determined using our analysis methodology, and who also have prior diagnostic CT scans of some time interval before treatment. This will allow us to determine the pre-treatment growth rate of these tumors and correlate this with elastometry to evaluate the ability of elastometry to identify tumors with a more aggressive growth rate. Tumor growth rate measurements are now in progress for this cohort of patients, and we anticipate it will be completed by the end of April 2015.

Key Research Accomplishments:

Our first aim was to develop and validate an automated software package for determining PN elasticity against a manual contouring method, and preliminarily assess its ability to distinguish malignant tissue by comparing the elasticities of malignant PN with those of the lung. This work is now completed and a manuscript detailing the methodology and the results was submitted to *Radiotherapy and Oncology*.

Reportable Outcomes:

The following abstract was accepted for POSTER presentation at ASTRO 2014:

1. Mohammadreza Negahdar, Billy W Loo, Maximilian Diehn, Lu Tian, Dominik Fleischmann, and Peter G Maxim, *"Automated Tool for Determining Pulmonary Nodule Elasticity to Distinguish Malignant Nodules,"* ASTRO 2014

The abstract is included in the 'Supporting Documentation' section.

A manuscript summarizing our initial validation was submitted to *Radiotherapy and Oncology*. It has been reviewed and we have submitted a second round of responses to reviewers' comments and anticipate acceptance as all concerns were addressed.

Conclusion:

We have successfully accomplished Specific Aim 1 of the proposed study. We now have functional software to process and analyze 4DCT images to distinguish malignant and benign PN. Despite setbacks in time because of upgrades of the small animal CT equipment, we have made substantial progress on Specific Aim 2 by completing gated image acquisition for both malignant orthotopic tumor and inflammatory talc nodules in the rat model. Remaining work includes increasing the sample size for each of these models, including a second benign nodule model with different stiffness, and analyzing the images. We have also now made significant progress on Specific Aim 3 by identifying the cohort of patients with potentially benign nodules, and also identifying a large cohort of patients with malignant nodules whose growth rate can be measured, and are actively performing the image measurements. As such, we believe we are on track to carry out the proposed research project within the period of the no-cost extension.

Supporting Data:

Abstract submitted to the Annual Conference of ASTRO (2014):

Automated Tool for Determining Pulmonary Nodule Elasticity to Distinguish Malignant Nodules

Purpose: To develop and validate an automated method of determining pulmonary nodule (PN) elasticity against a manual contouring method, and preliminarily assess its ability to distinguish malignant tissue by comparing the elasticities of malignant PNs treated with stereotactic ablative radiotherapy (SABR) with those of the lung.

Methods: We analyzed breath-hold images of 30 patients with malignant PNs who underwent SABR in our department. A parametric nonrigid transformation model based on multi-level B-spline guided by Sum of Squared Differences similarity metric was applied on breath-hold images to determine the deformation map. The Jacobian of the calculated deformation map, which is directly related to the volume changes between the two respiratory phases, was calculated. Next, elasticity parameter will be derived by calculating the ratio of the Jacobian of the PN to the Jacobian of a 1cm region of lung tissue surrounding the tumor (E-ROI) as well as the Jacobian of the whole lung (E-Lung).

Results: For the first group of 15 patients we evaluated the volumetric changes of PNs and the lung from the maximum exhale phase to the maximum inhale phase, whereas the reverse was done for the second group of 15 patients. For the first group, mean and standard deviation for E-ROI and E-Lung were 0.91±0.09 and 0.86±0.18, respectively, which was verified by the manual method. For the second group, E-ROI and E-Lung were 1.34±0.27 and 1.57±0.51, respectively. These results demonstrate that the elasticity of the PNs was less than that of the surrounding lung ($p<0.0037$).

Conclusion: We developed an automated tool to determine the elasticity of PNs based on deformable image registration of breath-hold images. The tool was validated against manual contouring. Preliminarily, PN elastometry distinguishes proven malignant PNs from normal tissue of lung, suggesting its potential utility as a non-invasive diagnostic tool to differentiate malignant from benign PN.